IN VIVO Screening Models of Anticancer Drugs

Natalia L. Blatt[1], Rimma N. Mingaleeva[1], Svetlana F. Khaiboullina[2], Alexander Kotlyar[3], Vincent C. Lombardi[2], Albert A. Rizvanov[1]*

[1.] Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, 420008, Russia
[2.] Department of Biochemistry and Molecular Biology, University of Nevada School of Medicine, University of Nevada-Reno, Reno, Nevada, 89577, USA
[3.] Department of Biochemistry & Molecular Biology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, 6997801, Israel
Albert.Rizvanov@kpfu.ru

Abstract: Animal models have been indispensable when conducting research to further the understanding of cancer biology and when developing anticancer drugs. This article presents an overview of the most commonly utilized animal models for preclinical screening of anticancer agents. These models can be roughly divided into two groups: models in which tumors are transplanted into mice, and models in which tumors develop *in situ*, either spontaneously or induced. Special attention is paid to the widely used subcutaneous xenotransplant and the orthotopic tumor models. We will also highlight the development and use of genetically modified mice.
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1. Introduction

In recent decades, our understanding of the molecular mechanisms of *in vivo* tumor formation and maintenance has grown significantly (Workman et al., 2010). This research has been driven by an increasing worldwide prevalence of cancer and the high mortality rate associated with most malignancies. Estimates as of 2004, suggest that over $200 billion had been invested, worldwide, on the war on cancer and the number of published articles relating to this research has surpassed 1.5 million (Leaf, 2004). This aggressive research and development campaign has resulted in a number of drugs for the treatment of malignant disease. One such example is the drug imatinib, which is sold under the trade name Glivec, has been effective for the treatment of chronic myeloid leukemia. However, despite the continuing development of numerous chemotherapeutic drugs, their effectiveness is still limited in that the majority of patients continue to die within five years of the commencement of treatment (Sharpless and Depinho, 2006). A number of reasons may contribute to the apparent lack of progress in the development of new efficacious anticancer drugs; however, most researchers consider the imperfection of existing *in vitro* and *in vivo* testing models as the principal obstacle.

Before any potential anticancer agent is subjected to human testing, the prospective drug undergoes a series of qualifying studies. Initially, primary *in vitro* screening is conducted, using the NCI60 human tumor cell line anticancer drug screening protocol, whereby a potential candidate is evaluated for its ability to inhibit the growth of tumor cells in culture. This modern pharmaceutical *in vitro* screening protocol was developed by the National Cancer Institute (NCI) and comprises a panel of 60 human tumor cell lines. Currently, the NCI60 is the most commonly used system for the preliminary screening of potential anticancer drugs (Shoemaker, 2006). Different cell and tissue culture systems for anticancer drug screening were extensively reviewed previously (Blatt et al., 2013). After the initial *in vitro* screening is completed, the candidate drug then moves on to *in vivo* animal testing. This phase of evaluation is critical for understanding the fundamental processes that support *in vivo* tumor development because tumor cells grown in culture are not necessarily analogous to those that develop in a human subject (Workman et al., 2010). There are several factors that contribute to this limitation; however, the two-dimensional environment imposed by standard tissue cultures methods is of critical importance. This issue is currently being addressed by the development of three-dimensional culture methods such as the multicellular spheroids models and Matrigel™ matrices; however, these systems only provide a modest improvement. *In vivo,* tumor cells grown in a three-dimensional natural environment interact with stromal elements and have access to vascularization. Therefore, even a three-dimensional cell culture system cannot fully reproduce the natural tumor microenvironment and, consequently, necessitate the use of *in vivo* systems.

The vast majority of *in vivo* cancer research is conducted using inbred laboratory mice (Workman et al., 2010); however, other animals such as dogs and primates are also often utilized for such reasons as to assess tolerability of dose and toxicity (Singh and Johnson, 2006). Mouse tumor models are utilized for a number of reasons including the similarity of human and mouse genomes; the low cost of housing and maintenance; the short gestation period and rapid reproduction rate; and the rapid growth rate of implanted tumors. These as well as other aspects make the inbred laboratory mouse an excellent tool for conducting cancer research and screening potential anticancer compounds. In this review, we discuss the advantages and disadvantages of in vivo cancer models existing to date.

2. Early *in vivo* test models

The first *in vivo* test models used for screening anticancer compounds were developed back in mid-1960s at the NCI. These syngeneic mouse leukemia models were initially grown as ascites tumors in mice (Teicher, 2006). The growth properties of these tumors were similar to those of bacteria *in vivo* and therefore, mathematical models that described the growth kinetics of bacteria were also utilized when describing the growth patterns of tumors were developed. Examples of such models include the leukemia cell lines P388 and L1210, which form tumor ascites after intraperitoneal implantation. These cell lines have been used extensively to screen compounds that inhibit fast-growing tumors and were instrumental in identifying several substances with clinically observed antitumor properties such as vinca alkaloids and nitrosourea (Damia and D'Incalci, 2009). Assessing the efficacy of anticancer compounds, using these models, is typically determined by calculating the ratio of the average increase in survival of the treated animal group over the control group (Teicher, 2006).

Models which have been shown to be effective for screening compounds that inhibit the growth of solid tumors include the murine melanoma cell line B16, grown in immunocompetent mice, and the cell line MX1; a human breast carcinoma line that develops in immunodeficient mice. In order to evaluate the effectiveness of compounds to inhibit the growth of solid tumors the tumor growth delay is determined, which is calculated as the difference in days for treated versus control tumors to reach a given size, typically 1 cm^3 (Teicher, 2006). Also developed at the NCI, the *in vivo* subrenal capsule system (SRC) utilizes a tumor biopsied directly from a cancer patient in order to assess the effectiveness of a potential anticancer compound (Maenpaa et al., 1988). This system exploits a tumor biopsied directly from the patient and implanted into an immunodeficient mouse kidney through an incision was made in the renal capsule of the animal (Fu et al., 1991). Effectiveness of tested substances is determined by inhibition of tumor growth in the kidney capsule. Although highly effective, this system is not widely used due to the technical complexity of surgical procedures and the need for multiple biopsies from the same individual to ensure consistency of the results thus hindering large-scale screening of compounds (2003).

3. The Hollow Fiber Assay

Currently, the most commonly used models for *in vivo* anticancer drug screening are xenotransplantation of human tumor to mice and the hollow fiber assay (HFA). Both models utilize the transplantation of tumor cells into immunodeficient mice. The xenotransplantation model has a number of drawbacks including the time required to screen prospective anticancer agents, the number of animals required and the cost involved. These issues prompted the development of the hollow fiber method at the NCI, as an additional qualifying step in drug development, bridging the gap between in vitro and in vivo xenograft screening of anticancer compounds (Shnyder et al., 2005). The purpose of this assay is to predict which compounds, that showed promise during the course of NCI60 human tumor cell line anticancer drug screening protocol, will show promise during *in vivo* xenograft screening (Damia and D'Incalci, 2009).

HFA was developed as a heterogeneous solid tumor model. The assay is based on the tumor cells ability to form tumors in hollow tubes consisting of polyvinylidene fluoride (PVDF). Within the tube a central core of necrotic cells is surrounded by a thin layer of living cells that are in contact with the wall of the hollow tube (Shnyder et al., 2005). HFA screening is carried out using a standard panel of 12 cancer cell lines (Table 1). Screening of specific compounds is also possible in other cell lines.

Table 1. Tumor cell lines, used in HFA

Cell line	Description
NCI-H23	non-small cell lung cancer
NCI-H522	non-small cell lung cancer
MDA-MB-231	breast cancer
SW-620	colon cancer
COLO 205	colon cancer
OVCAR-3	ovarian cancer
OVCAR-5	ovarian cancer
U251	encephaloma
SF-295	encephaloma
MDA-MB-435	melanoma
LOX	melanoma
UACC-62	melanoma

Cells to be used are cultured until they reach log phase growth (approximately 2 x 10^6 cells /ml), then the cell suspension is introduced into the tubes and incubated for 24-48 hours. The hollow tubes, which have an internal diameter of 1 mm and a length of 2 cm are permeable to molecules with a molecular weights up to 500 kDa and allow nutrients and potential anticancer drugs to enter the tubes and come into direct contact with the tumor (Damia and D'Incalci, 2009). PVDF is a biocompatible material that allows implanting of the hollow tubes into the immunodeficient mice, either subcutaneously or intraperitoneally (Benbrook, 2006). In a typical experiment, each animal receives three different implants, each containing a single tumor cell line. This reduces the number of animals necessary for the analysis, thus reducing cost. After 3 or 4 days post implantation, the drug to be tested is introduced into the animal through an intraperitoneal injection, and is continually delivered for the next 4 days. On the 6th day of treatment, the tube is removed and cell viability is determined by a modified MTT-test, which takes into account such *in vivo* parameters as pharmacokinetics, pH, and oxygen content within the tumor. Analysis of cell cycle, DNA damage and apoptosis induction can also be determined (Temmink et al., 2007). In general, the data derived regarding the tested substance in the intraperitoneal site is reflective of the overall tumor sensitivity to the drug. The obtained results are compared with *in vitro* results of the tested substance and if the substance also displayed activity in the subcutaneous area, it is reported to exhibit promising bioavailability and pharmacological properties (Phillips et al., 1998). Therefore, if the effectiveness of the compound is shown in both the intraperitoneal and subcutaneous sites, it is considered to be a promising anticancer agent and justifies further evaluation (Benbrook, 2006; Suggitt and Bibby, 2005).

This model's predictive ability has been evaluated by comparing the results of tested substances selected by HFA and those selected using xenotranplantants (Hollingshead et al., 1995; Lee and Rhee, 2005) as well as those selected using the *in vitro* screening panel NCI60 (Johnson et al., 2001). As expected, not all agents active in HFA, inhibit tumor growth of subcutaneous xenotranplantants. Although the HFA is useful tool for rapid identification of potential anticancer compounds, each promising compound must be thoroughly investigated in xenotransplantant models (2001).

One disadvantage of HFA is the spatial limitations of the model. Tumor growth is inhibited by the inside diameter of the tube and, therefore, to ensure credible experimentation, it is necessary to maintain tumor growth within the fiber close to its maximum. It is not recommended to make experiments during tumor growth rate deceleration. It is likely that this discrepancy can explain different results obtained between HFA and xenotransplantant models (Lee and Rhee, 2005). Another drawback is that the fiber wall is an artificial barrier between the tumor and its environment. This hampers the diffusion of large biomolecules, such as DNA and antibodies, which implies that the model is not amenable to studies using macromolecular agents or nanoparticles (Elliott and Yuan, 2010).

4. Human tumor mouse xenotransplantant models

In 1969 it was shown that human tumor cells, cultured *in vitro*, could be implanted into immunodeficient mice (Rygaard and Povlsen, 1969). The normal adaptive immune responses associated with foreign tissue rejection, such as killer T cells inducing apoptosis of target cells and humoral immune responses mediated by antibody-secreting activated B cells, is suppressed and the tumor is not rejected. When the tumor reaches a given size, introduction of a potential anticancer drug is made and the efficacy of the drug is determined by changes in the tumor size. If a potential candidate shows promising results, a series of experiments can be conducted out to optimize the drug dosing and determine the efficiency of the substance in order to reduce its toxicity by adjusting the dose and mode of application (2011).

The simplest model of xenotransplantation is achieved by the subcutaneous introduction of tumor cells. This model allows the rapid quantification of a compound's anticancer properties as well as its toxicity (Peterson and Houghton, 2004). The allure of the model is that a tumor is developing develops on the surface of the animal, thereby allowing the tumor volume to be easily measured and additionally, facilitates the facile observation disease dynamics (Talmadge et al., 2007). Subcutaneous xenotransplantants have been used successfully to predict clinical outcomes of substances, for which the activity is not tumor-dependent, such as cyclophosphamide (Kelland, 2004). Using subcutaneous xenotransplantant models, these substances were shown to be efficacious in treating rhabdomyosarcoma and adenocarcinoma human colon cancers in clinical trials (Peterson and Houghton, 2004). Because the xenotransplantation model must be conducted using immunodeficient mice, the tumor microenvironment afforded by this system is not necessarily a precise representation of the naturally occurring microenvironment (Richmond and Su, 2008). Additionally, immunodeficient mice are not suitable for testing substances that interact with or modulate the immune system (Gordon and Khanna,

2010). However, despite its many shortcomings and limitations, the xenotransplantation model is a pillar of preclinical anticancer drug testing (Singh and Johnson, 2006).

An obvious limitation to the subcutaneous transplantation system is that the tumor cells are grown in a tissue microenvironment that may be substantially different when compared to the environment they experience when naturally occurring in a human subject (Fu et al., 1991). To address this limitation, the orthotopic xenotransplantation model was developed which more closely simulates the morphology and growth properties a tumor experiences in its natural microenvironment. In this model, a subject`s tumor cells are transplanted into the orthotopic area of a mouse; for example, colon cancer cells are transplanted into the intestinal wall of an immunodeficient mouse (Benbrook, 2006). The orthotopic introduction of tumor cells increases the frequency of metastases, which are rare in the subcutaneous xenotransplantation model (Hoffman, 1999); however, metastases observed as a result of orthotopic xenotransplantation exhibit properties similar to those observed in human subjects (Fu et al., 1991). Additionally, the orthotopic xenotransplantation model allows one to demonstrate the antitumor and antimetastatic efficacy of a given substance. For instance, this model has been used to identify drugs with angiogenic properties such as batimastat and TNP-470 (Hoffman, 1999).

An impediment to use of orthotopic xenotransplantation in large-scale studies is the need for advanced surgical procedures for placement of tumor, which requires a high level of technical skills (Benbrook, 2006). The time required for such surgical procedures limits the number of mice that can be practically sampled during a single experiment (Teicher, 2006). Additionally, internal growth of tumors formed by orthotopic transplantation and their response to the tested substance is difficult to track over time (Teicher, 2006). Moreover, it is necessary sacrifice the animal order to investigate the tumor, further adding to the cost of an already expensive experiment (Suggitt and Bibby, 2005).

Retrospective studies have shown that the subcutaneous xenotransplantation model may produce spurious results, incorrectly supporting the efficiency of drugs that later failed during clinical trials (Kerbel, 2003). A striking example of this was in the evaluation of the antitumour agent aminokaptotetsin 9 (9-AC). In 1989, the results of an impressive preclinical study based on a subcutaneous xenotransplantation model of colon cancer using immunodeficient mice were report (Giovanella et al., 1989). Later it was again erroneously reported that the compound is effective in other human tumors (Pantazis et al., 1992; Pantazis et al., 1993). The results of these studies prompted the NCI to include 9-AC in the priority list of compounds for clinical trials. The first phase of clinical trials was initiated in 1993; however, the drug failed the second phase, when lung and colon tumors did not respond to the treatment (Takimoto, 2001; Vokes et al., 1998; Wilson et al., 1998). Comparing the pharmacokinetics and pharmacodynamics of 9-AC in preclinical experiments and early clinical trials revealed possible reasons for these disappointing results. However, the ultimate conclusion made, was that it is necessary to process and evaluate the results of preclinical studies and early clinical trials much more judiciously (Takimoto, 2001).

Clearly, it is necessary to better understand the limitations of existing models of preclinical screening so these important tools can be improved upon. As an example, it has been shown that the use of cell lines for xenotransplantation does not accurately simulate the *in vivo* tumor microenvironment. Xenotransplantants that utilize tumor biopsies from human subjects are more predictive of clinical responses (Hoffman, 1999). Typically, such xenotransplantants more closely resemble natural tumors in architecture, cell morphology and molecular characteristics, whereas xenotransplantants derived from cell lines often present a homogeneous and undifferentiated histology, as well as display resistance to many anticancer drugs (2001; Gordon and Khanna, 2010). These characteristics are likely the result in the selective pressures experienced during prolonged *in vitro* culturing. In cases where transition of the implanted biopsy is problematic, it has been proposed to significantly increase the number of cell lines for which the drug is tested. This will enable the evaluation of a more comprehensive spectrum of genetic changes that are associated with clinically observed tumors (Sharpless and Depinho, 2006). Although more accurately representing the naturally occurring tumor microinviroment, the use of orthotopic xenotransplantants as a model of metastatic tumors is often not adequate. Although the challenges associated with treatment of tumors with extensive metastases are well known, the most effective substances showing success in preclinical testing are employed during the small metastases stage. (Kerbel, 2003). Thus, in order to more closely mimic natural conditions, the testing of substances on orthotropic transplants should begin at the stage of macroscopic metastases.

Finally, special attention should be paid to the inconsistencies between humans and mice when considering the dose of a potential drug under study. For mice, the dose of most chemotherapeutic drugs

necessary for a proper clinical effect typically exceeds that needed for humans by 4 to 5 times (Gordon and Khanna, 2010; Kerbel, 2003). Therefore, the amount of drug sufficient for detecting a measurable effect in mice is often not enough to produce clinical effect on humans. However, for tumors growing as murine xenotransplantants, it has been shown that a clinically relevant dose in mice is often similar to that observed for primary human tumors (Sharma et al., 2010).

5. Autochthonous tumor models

Autochthonous tumors either arise spontaneously or can be induced by carcinogens or other chemical, viral, bacterial, or physical triggers (Workman et al., 2010). Their histological characteristics are more like those of human tumors than of xenotransplantants. Autochthonous tumor models allow one to investigate new molecular targets for preventive chemotherapy by studying processes of mutations, oxidative stress and inflammation, which occur during tumor formation. For example, autochthonous mammary tumors induced by dimethylbenzanthracene have proven to be useful in identification of new therapeutic agents (Sugamata et al., 1999). Workman et al. (Workman et al., 2010) recently reviewed the list of available primary tumor models emphasizing their respective advantages and disadvantages. Although these models may be used to identify new molecular targets, they are rarely used to directly test the antitumor agents. Due to challenges in imaging of tumors growing within internal organs, as well as the fact that tumor formation is preceded by a long period of carcinogen treatment, after which the tumors develop asynchronous, these models are impractical for high-through put screening (Teicher, 2006; 2001).

6. Genetically engineered mouse models

In contrast to immunodeficient mice, tumors grown in genetically engineered mouse (GEM)-models are affected by the immune system and interact with stroma; therefore, making it possible to test drugs which targets reside within an immunocompetent tumor microenvironment (Singh and Johnson, 2006; Damia and D'Incalci, 2009). When the first GEM-tumor models appeared in the 1980s, it was shown that to induce turmorgenesis in mouse cells it was enough to introduce expression cassettes containing a large T-antigen under the control of the promoter and enhancer regions of simian vacuolating virus 40 genes (Palmiter et al., 1985). By using tissue-specific promoters to regulate antigen expression, it is possible to obtained specific types of tumors (Hanahan, 1985). In order to activate the transgene expression in specific tissues one should also use the mouse viral regulatory elements. For example, transgene delivery into mouse cells, the expression of which is controlled by a long terminal repeat of mouse mammary tumor virus, leads to breast tumor development, with morphology and gene expression patterns similar to that of natural human breast tumors (2011; Stewart et al., 1984). However, the cellular introduction of transgenes does not give rise to all types of tumors; formation of some requires silencing of tumor suppressor gene expression. The process of homologous recombination can allow one to delete, move or introduce mutations into a gene in mouse embryonic stem cells and silence these genes. For example, mouse pituitary adenoma is produced through removal of one of the alleles of tumor suppressor gene Rb (2011).

Tumors that arise from the spontaneously transformation of cells typically do so during the animal's juvenile or adult stage of development. By utilizing the GEM-model it is possible to change the expression of the genes of interest at the embryonic stage of development. This, in turn, leads to a change in the expression of most, if not all progeny cells observed in the adult. Currently, differences between embryo and adult gene function and expression patterns are not fully understood; therefore, to more closely simulate human tumors is it sometimes preferable to introduce changes into some cells of the adult animal (2011). By using methods to control recombinant genes expression, it is possible to activate a given gene in a tissue of interest and in the desired time interval. For example, the Tet-on inducible system (Clontech, Inc, U.S.A.), expression of a desired transgene previously introduced into the cell, is activated only after low doses of doxycycline is given to the animal (Kistner et al., 1996). Expression activators can also be delivered as part of a genetic construct. For instance, in lung tissue, the transgene expression-control of an inducible Cre-Lox, can be activated through intranasal introduction of adenovirus particles expressing the recombinase gene Cre (Singh and Johnson, 2006).

In the last decade, by modifying genes crucial for the development of specific types of tumors, researchers have developed mouse models of lung, breast, colon, ovary, pancreas and prostate cancers. For instance, the GEM-model of non-small cell lung carcinoma is obtained through simultaneous activation of protooncogen K-RAS and deactivation of Rb and p53. Several GEM-models with overexpression of c-Myc, cyclin D1, Her2 and Wnt-1 oncogenes were developed to simulate breast cancer (Singh and Johnson, 2006). Angiogenesis inhibitors are often tested on rat insulin promoter 1 (Rip1)T-antigen 2 (Tag2)-transgenic mouse model of pancreatic β-cell lymphoma. In this model of multistage insulinoma, the rat insulin promoter gene

Rip1, is specifically activated in pancreatic cells, and induces expression of a large tumor antigen of SV40 virus Tag in β-cells of the islets of Langerhans (Yundan, 2008). TRAMP mouse model is another widely used system for the induction of prostate transgenic adenocarcinoma. The TRAMP-model has given rise to several transplantable tumor lines, used to study tumor angiogenesis, immunotherapy, and gene therapy (Teicher, 2006). Transgenic mice have also been useful to explore new properties of known drugs, such as those that are employed in preventative chemotherapy and are administered for a long time to prevent tumor growth. For instance, it has been shown that a daily dose of 20 mg/kg of the NSAID R-flurbiprofen significantly reduced the occurrence of prostate primary tumors and the frequency of metastases in TRAMP-mice (2001; Nguewa and Calvo, 2010). A comprehensive description of currently available GEM-models is provided in the excellent review of Singh et al. in 2006 (Singh and Johnson, 2006).

An animal's life span and tumor volume is not always a reliable indicator of the efficacy of an antitumor agent. The tumor volume endpoint does not allow one to detect small changes in the tumor's mass or micrometastases. In addition, in is necessary to sacrifice an experimental animal in order to evaluate tumor growth in internal organs, making not possible to monitor the tumor growth dynamics. GEM-models with reporter proteins enable to overcome these limitations (Chishima et al., 1997; Hollingshead et al., 2004). Such models use tumor cell lines that carry bioluminescent or fluorescent reporter proteins genes such as firefly luciferase gene (LUC) or green fluorescent protein (GFP) of jellyfish. Their *in vivo* expression in tumor cells allow one to visualize tumor progression, to monitor its response to anticancer agent, and to visualize internal metastases and tumor nodules (Hoffman, 1999; Hollingshead et al., 2004; Hoffman, 2005).

The advent of transgenic organisms expressing reporter protein genes has made it possible to distinguish normal cells of a host organism from implanted tumor cells (Hoffman, 2005). For example, if cells expressing the red fluorescent protein (RFP) gene are implanted withing a GFP-expressing tumor, mouse recipient cells will emit red light, while tumor cells will show green fluoresce. This delineation allows one to easily identify tumor-induced responses (Teicher, 2006). It is noteworthy that the technology of fluorescent or bioluminescent tumor imaging is still at its infancy. Clinical visualization tools adapted for small laboratory animals such as micropositron emission tomography, ultrasound, magnetic resonance imaging and X-ray microcomputer tomography *in vivo*, have great future potential for monitoring internal processes, including tracking the growth of tumors in these models (Heyn et al., 2006; Li et al., 2006; Memon et al., 2009; Wu et al., 2005).

Despite the temptation to use GEM as a preclinical screening model, its predictive properties are still inconsistent. Albeit, the activity of some substances tested using this model has been shown to be similar to their activity in clinical trials. For example, despite its high efficiency in xenotransplantants, troglitazone did not show any activity in GEM-models or in clinical trials, which suggested GEM-models to be predictive, unlike xenotransplantants. However, when other substances, like farnesyl transferase inhibitors were tested, GEM and xenotransplantant models exhibited tumor growth inhibition; however, they did not show the expected activity in clinical trials (2011).

Besides predictive uncertainty, GEM-models have following disadvantages. Genetically engineered mice are rather expensive and difficult to generate. Currently, one can quickly obtain a large number of genetically modified mice that are in one stage of development, using *in vitro* fertilization technology. However, these mice and those obtained through traditional crossing often produce spontaneous and multifocal tumors and display variable tumor growth (Damia and D'Incalci, 2009). In addition, there is a paucity of inducible GEM-models for most types of tumors and their analysis requires the use of expensive photo-sensitive detection systems (2011). Fluorescence intensity or bioluminescence reporter protein in different tissues and tumor types often varies (Singh and Johnson, 2006; Teicher, 2006). Moreover, the use of these mice are generally protected by patents (Brown, 2000). These issues, as well as others complicate anticancer agent testing.

Although GEM-models may not replace xenotransplantants, they can play an intermediary role between xenotransplantant screening and clinical trials. Tumors obtained from genetically-modified mice can be isolated and cultured in vitro. The advantage of these cells is that their transformation took place in a natural microenvironment and is influenced by the immune system. Such tumors when implanted subcutaneously or orthotopically into immunocompetent mice allow one to more fully investigate the effect of anticancer agents (2011). GEM-models afford a unique opportunity to characterize differences in cell lines and natural tumors by comparing their genotypes and phenotypes. It becomes possible to restore tumor heterogeneity through in vitro multistep cancerogenensis. It is logical to assume that cell lines possessing changes similar to those of tumor cells *in vivo*, can be used for the initial screening of drugs (Singh and Johnson, 2006). The full potential of these models is yet to be realized.

7. Conclusion

Currently, there are a limited number of models designed for preclinical in vivo drug screening. Hollow fiber and xenotransplantant models have been utilized extensively; however, they are limited in their abilities and do not led themselves well to the high throughput screen studies necessary to evaluate antitumor drugs. Although models utilizing genetically modified mice offer an attractive and promising alternative, they fail in many respects compared to xenotransplantant models.

A critical question that needs to be considered is why many substances that appeared promising in preclinical screening, do not exhibit the desired properties in vivo? There are several possible explanations for this. First, the discrepancy may stem from incorrect assessment misinterpretation of results of the animal testing. When developing "targeted" drugs the target choice is critical as well as the method by which the effect will be evaluated. Often, an inadequate understanding of tumor biology brings inefficient compounds to the preclinical screening stage. Moreover, differences between mouse and human genomes raise the question of relevancy to use rodents in such studies. When using mouse models, a potential anticancer drug is tested in the environment different from that in clinical subjects. For example, in contrast to humans, most murine cells have functionally active telomerase (Prowse and Greider, 1995; Rangarajan and Weinberg, 2003). Changes in certain genes and their associated pathways, such as TP53, Rb and Ras, lead to different pharmacological effects in mouse and human cells (Rangarajan and Weinberg, 2003). In many cases, mice withstand higher drug concentrations than people. For instance, mouse bone marrow is less sensitive to many cytotoxic agents (Gordon and Khanna, 2010; Teicher, 2009). Thus, the results of laboratory testing carried out on syngeneic or xenogeneic tumor immunized mice cannot be directly extrapolated to humans.

A personalized medicine approach which uses biopsied tissue in lieu of tumor cell lines to screen antitumor substances may draw us closer to actual clinical conditions. Development of GEM-inducible models and models with multiple genetic alterations may also bring us closer and make GEM-model more predictive.

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Corresponding Author:
Albert A. Rizvanov, Ph.D., Dr.Sci.
Chairperson of the Department of Genetics
Institute of Fundamental Medicine and Biology
Kazan (Volga Region) Federal University
Kremlevskaya st 18, Kazan, Russia, 420008
E-mail: Albert.Rizvanov@kpfu.ru

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